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Electrochemical protein cleavage in a microfluidic cell for proteomics studies

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Abstract

Electrochemical protein digestion prior to mass spectrometric analysis is a purely instrumental approach to protein identification, offering reduced consumption of chemicals and shorter analysis times compared to the use of enzymes and chemical cleavage agents. Here we demonstrate the possibilities of site-specific peptide bond cleavage and disulphide bond reduction in a microfluidic electrochemical cell. The use of microfluidics in this context is beneficial for increased electrochemical cleavage yields, small sample volumes and the possibility of rapid on-line analysis, thereby providing a versatile tool for routine proteomics studies.

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Keywords: Peptide bond cleavage; Disulphide bond reduction; Electrochemical protein digestion; Microfluidic electrochemical cell; Electrochemistry/Mass spectrometry; Proteomics

1. Introduction

Protein cleavage prior to mass spectrometric analysis is an established approach to bottom-up protein identification, currently relying on trypsin as the workhorse to generate proteolytic peptides. Electrochemical protein cleavage has been investigated as an instrumental alternative, which occurs specifically at the C-terminal side of tyrosine and tryptophan residues [1]. If no measures are taken, electrochemically generated peptide fragments can be linked together

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by disulphide bonds between cysteine residues, thereby hampering protein identification. Also these bonds can be cleaved electrochemically, as an instrumental alternative to the commonly used chemical reducing agents [2]. We have exploited the benefits of miniaturization to develop a microfluidic electrochemical cell with integrated boron doped diamond working and counter electrodes to achieve high electrochemical conversion efficiencies while consuming small amounts of analyte [3,4]. In the most recent developments, we employ these devices to cleave peptide and disulphide bonds and identify proteins in a purely instrumental fashion.

2. Methods

Bovine insulin was prepared at a 10 µM concentration in 85/10/5 (v/v/v) water/acetonitrile/formic acid. The analyte flow over the working electrode was 1 µL/min, while a constant potential of 2000 mV (peptide bond cleavage) or -2000 mV (disulphide bond cleavage) was applied vs. an integrated platinum pseudo-reference electrode. Reaction product mixtures were collected and analyzed by LC/ESI-MS/MS. Design and characterization of the microfluidic electrochemical cell and protein cleavage experiments are described in detail in reference [4].

3. Results

Insulin, which has 4 tyrosine residues, was cleaved electrochemically at these sites by applying a potential of 2000 mV. Off-line LC/MS analysis revealed that all 3 cleavage products reported in the literature were generated [5], which were confirmed by MS/MS. Extracted ion chromatograms are shown in figure 1A. Next, the three disulphide bonds (two intermolecular and one intramolecular) were cleaved electrochemically by applying a potential of -2000 mV. LC/MS analysis revealed the presence of the expected separated polypeptide chains (see figure 1B).

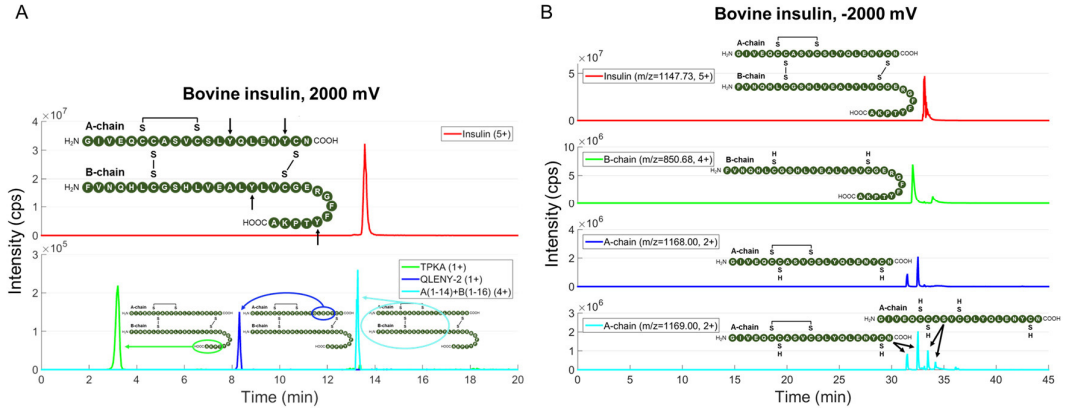

Figure 1: Electrochemical cleavage of insulin in a microfluidic electrochemical cell using constant potentials followed by LC/MS analysis. A:Application of a positive potential of 2000 mV results in peptide bond cleavage. Extracted ion chromatograms are shown for intact insulin and three cleavage products. Arrows in the inset point at the four tyrosine residues. B: Application of a negative potential of -2000 mV results in disulphide bond cleavage. Extracted ion chromatograms are shown for intact insulin, the separated B-chain, and the separated A-chain. The latter was detected with intact and with reduced intramolecular disulphide bond.

4. Conclusions

These experiments demonstrate the potential of this microfluidic device to be used as a purely instrumental alternative to existing (enzymatic or chemical) protein cleavage methods currently in use for protein identification, with the benefits of full control over reaction conditions, reduced sample handling and faster analysis. Future work will be directed at increasing cleavage yields and combining electrochemical peptide bond and disulfide bond cleavage in a single electrochemical digestion protocol, thus providing a powerful platform for proteomics studies.

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